Development of animal-free hydrogel as Bio-ink for Cartilage Tissue Reconstruction

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Introduction

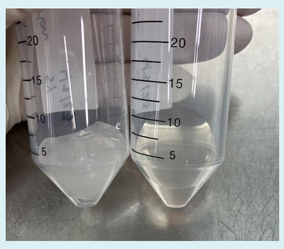
Three-dimensional bioprinting holds promise in the anatomical fabrication of lost tissues and organs. Cell-laden hydrogels have been widely used as bio-inks, extruded through a nozzle of 3D bioprinter to form the desired shape layer-by-layer. However, the major challenge in 3D bioprinting is finding functional biomaterials to develop bio-inks, besides animal-based biomaterials, such as gelatin and collagen.

The amine-hyaluronic acid ($HA\text{-}NH_2$) was covalently crosslinked with the aldehyde-alginate (Alg-CHO). Once $HA\text{-}NH_2$ and Alg-CHO solutions combine, by varying volume ratios, gelation is initiated through a Schiff's base reaction.

The goal of this study was to investigate how volume ratios of $HA\text{-}NH_2$ and Alg-CHO had impacts on the printability and biodegradability of the HA-Alg hydrogel and its potential use in the chondrogenic differentiation of mesenchymal stem cells (hMSCs).

The HA-Alg hydrogel made from equal volumes of $HA\text{-}NH_2$ and Alg-CHO exhibited shear-thinning behaviours, which are essential features of a printable bio-ink. We demonstrated cartilage tissue formation by encapsulating hMSCs in the HA-Alg hydrogel for 4 weeks.

The results suggest that the HA-Alg hydrogel can be used as a printable biomaterial for the extrusion-based 3D bioprinter. The HA-Alg hydrogel promoted cartilage tissue development and potentially supported other tissue formation due to its tailorable mechanical and degradable properties.

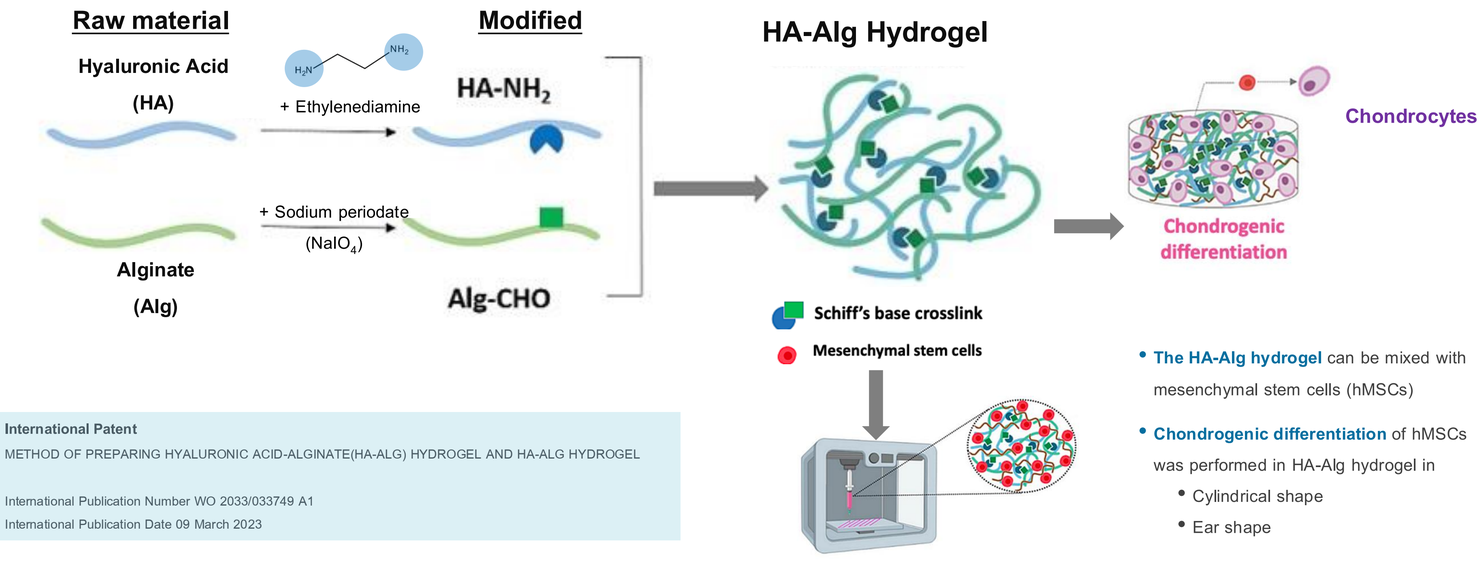


$HA\text{-}NH_2$ solution Alg-CHO solution

Materials and Methods

Preparation of HA-Alg hydrogel

- The carboxyl groups (-COOH) groups on **hyaluronic acid (HA)** were replaced by ethylenediamine resulting in hyaluronic acid-Amine ($HA\text{-}NH_2$)
- **Alginate (Alg)** was oxidized by Sodium periodate resulting in breaking the bond between C-2 and C-3 and aldehyde group (-CHO) formation



International Patent
METHOD OF PREPARING HYALURONIC ACID-ALGINATE(HA-ALG) HYDROGEL AND HA-ALG HYDROGEL

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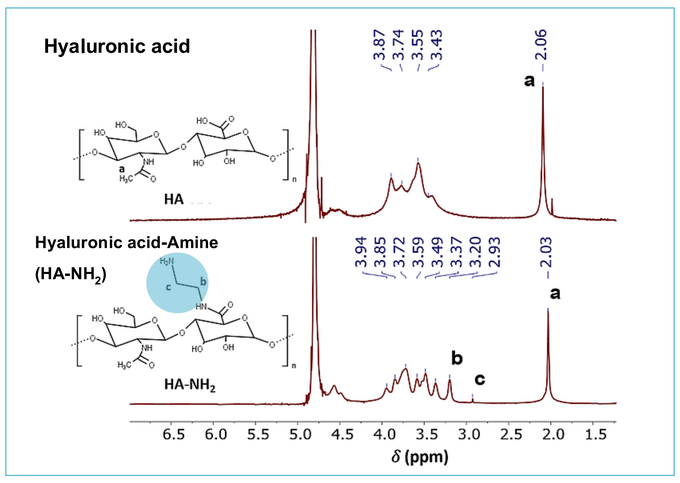
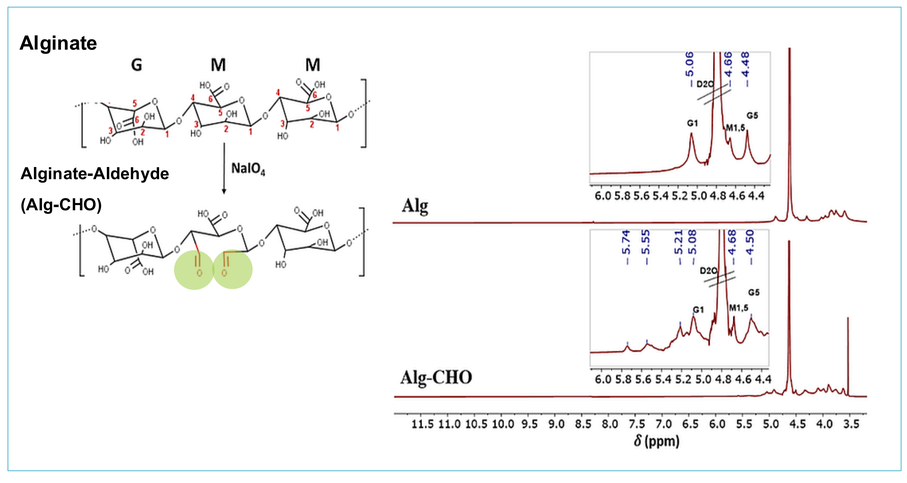
- **The HA-Alg hydrogel** can be mixed with mesenchymal stem cells (hMSCs)
- **Chondrogenic differentiation** of hMSCs was performed in HA-Alg hydrogel in
  - Cylindrical shape
  - Ear shape

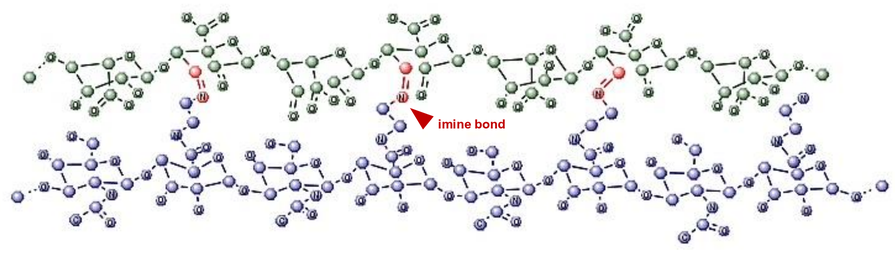
Results

NMR Analysis of Hyaluronic acid and Alginate

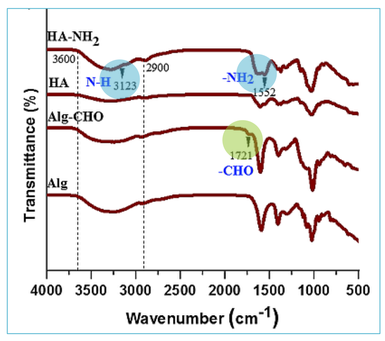




Imine bond formation by Schiff's base reaction



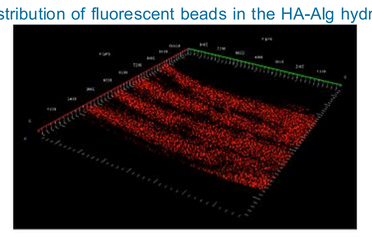
FTIR analysis of $HA\text{-}NH_2$ and Alg-CHO



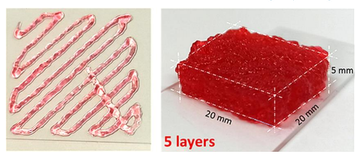
$HA\text{-}NH_2$ and Alg-CHO at volume ratio of 1:1

Printability of the HA-Alg hydrogel

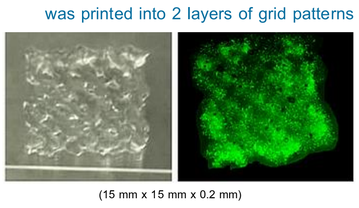
A distribution of fluorescent beads in the HA-Alg hydrogel



3D printed construct of the HA-Alg hydrogel



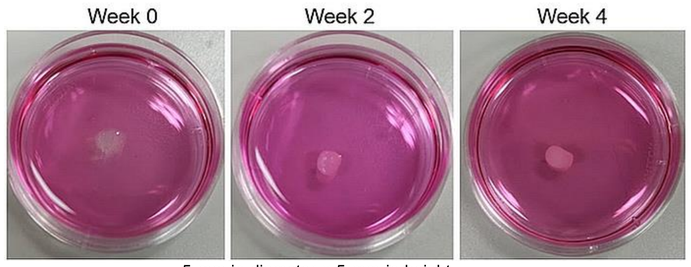
Human mesenchymal stem cells mixed with HA-Alg hydrogel was printed into 2 layers of grid patterns



(15 mm x 15 mm x 0.2 mm)

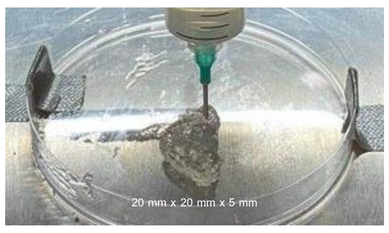
Chondrogenic differentiation of hMSC in HA-Alg hydrogel

The cells/hydrogel construct transformed from a transparent color to a white opaque construct in 4 weeks



5 mm in diameter x 5 mm in height

HA-Alg hydrogel was 3D printed in to ear-shape structure



Discussion

- Hydrogel is commonly used as a bio-ink because of its hydrated environment, which is similar to native tissue, excellent biocompatibility and adjustable viscosity.
- We aimed to develop the hydrogel and bio-ink, comprising bacterial hyaluronic acid (HA) and alginate (Alg), which are animal-free biomaterials.
- The gelation of the HA-Alg hydrogel was induced by covalent crosslinking via imine bonds, which was self-healing, temperature-independent and demonstrated competitive advantages over other options for material handling.
- Hyaluronic acid provides a biological cue in the HA-Alg hydrogel system, as one of the principle extracellular matrix components . However, it is difficult to form gel alone because of low mechanical properties and rapid degradation.
- To leverage the use of hyaluronic acid in tissue-engineering applications and bio-ink, Alginate was incorporated into HA-Alg hydrogel to improve the structural stability of hyaluronic acid.
- Crosslinking between $HA\text{-}NH_2$ and Alg-CHO resulted from Schiff's base reaction. The HA-Alg hydrogel with tuneable properties, by varying volume ratios of $HA\text{-}NH_2$ and Alg-CHO, could expand the range of applications to encapsulate different cell types and differentiate specific tissues.

Conclusion

- This study reports on a novel composite hydrogel made from $HA\text{-}NH_2$ and Alg-CHO.
- To initiate the gelation process, $HA\text{-}NH_2$ was crosslinked with Alg-CHO with different volume ratios, resulting in the formation of imine bonds (C = N).
- The HA-Alg hydrogel with volume ratios of 1:1 exhibited a matrix capable of supporting cartilage tissue formation.
- The versatility of the HA-Alg hydrogel was demonstrated by tailoring its mechanical properties, degradation time, and viscosity.
- Finally, shear- thinning behaviour and consistent biological properties (cell viability/ proliferation) of the HA-Alg hydrogel suggested that these key features contribute to extrusion-based 3D bioprinting technology.

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Intellectual property strategy
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- Thailand Research and Innovation Utilization Promotion Act (TRIUP Act)